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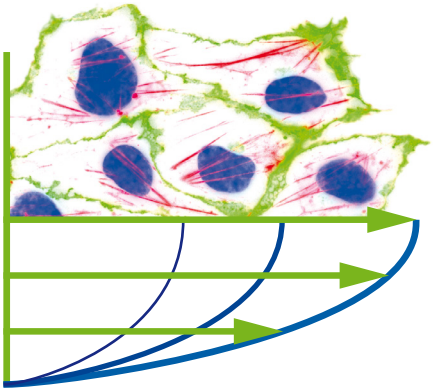
cells in focus

International **Cell Culture Under Flow** Meeting 2020



February 18–19 · Biomedical Center Munich, Germany

Abstract Book

Focus of the Meeting

The first international "Cell Culture Under Flow" meeting will bring together scientists from a variety of fields (e.g., vascular research, immunology, barrier function), who cultivate cells under shear stress.

Participants will have the chance to present their most recent work. In addition, both young and experienced scientists will have the opportunity to network and discuss future objectives of this field of research. Abstracts will be selected for oral presentation and posters.

Five sessions will cover the following topics:

- Blood cells under flow
- Endothelial/epithelial barrier function
- Microvascular mechanisms
- Transendothelial and interstitial migration
- Cells under flow – new technologies and applications

February 18

Registration and Reception

12:30	10 min	Welcome: Markus Sperandio (LMU Munich) / Roman Zantl (ibidi GmbH)
12:40	35 min	Keynote: On-chip leukocyte phenotyping *Scott Simon (University of California, Davis, USA)* *Chair: Markus Sperandio*

Session 1: Blood Cells Under Flow

13:15-15:00 Chair: Jaap van Buul (University of Amsterdam, The Netherlands)

13:15	35 min	Keynote: tba *Barbara Walzog (LMU Munich, Germany)*
13:50	35 min	Keynote: Simplifying the complexity of leukocyte recruitment - *in vitro* flow chambers complement intravital microscopy *Roland Immler (LMU Munich, Germany)*
14:25	15 min	A new microfluidic model that allows monitoring of complex vascular structures and cell interaction in a 3D biological matrix *Christian van Dijk (UMC Utrecht, The Netherlands)*
14:40	15 min	Maternal platelet interactions with fetal extravillous trophoblast cells *Jacqueline Guettler (Medical University of Graz, Austria)*

14:55	65 min	Coffee break with pastries, Poster Session I (odd numbers)

Session 2: Endothelial/Epithelial Barrier Function

16:00-17:55 Chair: Julia Mack (University of California, Los Angeles, USA)

16:00	35 min	Keynote: Lymph flow maintains the endothelium barrier integrity in lymphatic collecting vessels *Amélie Sabine (University of Lausanne, Switzerland)*
16:35	35 min	Keynote: Impact of shear stress on endothelial function *Henning Morawietz (University of Technology Dresden, Germany)*
17:10	15 min	Shear stress enhances stem cell characteristics, stratification and barrier function in human corneal epithelial cells *Sophia Masterton (Trinity College Dublin, Ireland)*
17:25	15 min	Cell culture models to study endothelial activation under septic conditions *Tanja Eichhorn (Danube University Krems, Austria)*
17:40	15 min	A human blood-brain-barrier *in vitro* model to investigate the role of FOXF2 in cerebral small vessel disease *Judit González-Gallego (Klinikum der Universität München, Germany)*

17:55	20 min	Drinks
18:15	35 min	Keynote: Bioimaging tools in experimental medicine *Steffen Dietzel (LMU Munich, Germany)* *Chair: Markus Sperandio*
18:50	open	Wine and fingerfood

February 19

Session 3: Microvascular Mechanisms

8:30-10:25 **Chair: Henning Morawietz (University of Technology Dresden, Germany)**

8:30	35 min	Keynote: Flow induced polarization of endothelial cells *Julia Mack (University of California, Los Angeles, USA)*
9:05	35 min	Keynote: Microvesicle-endothelial interactions under shear stress may provide clues for blood vessel pathophysiology *Allan Kiprianos (Imperial College London, UK)*
9:40	15 min	Dual chamber design for accessible, bidirectional perfusion of whole blood vessels *Julia Tarnick (University of Edinburgh, UK)*
9:55	15 min	Increased vascular permeability of human iPSCs-derived endothelial cells with mutation in HNF1A used as a model of maturity-onset diabetes of the young *Neli Kachamakova-Trojanowska (Jagiellonian University, Poland)*
10:10	15 min	Analyses of autologous endothelial colony forming cells under different shear stress applications *Xenia Kraus (Leibniz University, Hannover, Germany)*

10:25	35 min	Coffee break with cookies

Session 4: Transendothelial and Interstitial Migration

11:00-12:25 **Chair: Scott Simon (University of California, Davis, USA)**

11:00	35 min	Keynote: How the endothelium drives leukocyte transendothelial migration *Jaap van Buul (University of Amsterdam, The Netherlands)*
11:35	35 min	Keynote: Leukocyte navigation in 3D microenvironments *Jörg Renkawitz (LMU Munich, Germany)*

12:10	15 min	Smooth muscle cell migration and polarization in response to vascular injury *Shiemaa Khogali (University of Toronto, Canada)*

12:25	65 min	Lunch with sandwiches, Poster Session II (even numbers)

Session 5: Cells Under Flow – New Technologies and Applications

13:30–14:50 Chair: Amélie Sabine (University of Lausanne, Switzerland)

13:30	35 min	Keynote: Cultivation and high-quality imaging of cell aggregates under flow *Jan Schwarz (ibidi GmbH, Germany)*
14:05	15 min	Treatment of *Staphylococcus aureus* biofilms under flow using ultrasound and vancomycin-targeted microbubbles *Joop J.P. Kouijzer (Erasmus MC, The Netherlands)*
14:20	15 min	Engineered skeletal muscle from human induced pluripotent stem cells: potential of flow-based muscle modeling to study neuromuscular diseases *Masatoshi Suzuki (University of Wisconsin, Madison, WI, USA)*
14:35	15 min	Interrogating the biochemical status of cells under flow using Raman-laser-trapping, an innovative analytical tool for cell analysis *Hesham K. Yosef (CellTool GmbH, Tutzing, Germany)*

14:50	20 min	Closing Remarks, Young Scientist Award

Keynote 1

On-chip leukocyte phenotyping

Scott Simon

Department of Biomedical Engineering, University of California, Davis, USA

Biomedical engineers and cell biologists have collaborated to develop and apply a variety of optical and electronic technologies to spy on leukocytes as they ply their sensory systems, braking mechanisms, and locomotory machinery in their journey from the blood stream into the inflamed tissue during host defense and in auto-immune disease. We describe the application of novel PDMS-based microfluidic devices for imaging leukocyte interaction with biological substrates. Computational fluid dynamics provide a means of modeling the shear stress and the trajectory of leukocytes within parallel plate flow channels at a micron length scale. Microfluidic techniques employing transparent channels combined with fluorescence microscopy facilitates real-time imaging of leukocyte capture dynamics on recombinant adhesion molecules or inflamed endothelial monolayers. This approach has yielded a detailed temporospatial model of the multistep process of leukocyte recruitment. We will present a model of neutrophil recruitment that defines the superposition between biochemical signaling due to chemokine receptor ligation and mechanotransduced inside-out signaling via tension on selectin and integrin receptors.

Keynote 2

tba

Barbara Walzog

LMU Munich, Germany

tba

Keynote 3

Simplifying the complexity of leukocyte recruitment - *in vitro* flow chambers complement intravital microscopy

Roland Immler

Institute for Cardiovascular Physiology and Pathophysiology, Walter Brendel Center for Experimental Medicine, Biomedical Center, Munich, Germany

Neutrophils constitute the first line of defense during acute inflammatory processes fighting invading pathogens. Their recruitment from the blood stream to the sites of insult follows a well-defined, multistep cascade, starting with neutrophil tethering and rolling along inflamed postcapillary venules. Interactions of neutrophils with the endothelium activates the immune cells, resulting in neutrophil arrest followed by intraluminal crawling and subsequent transmigration into the surrounding tissue. Intravital microscopy offers the opportunity to investigate neutrophil recruitment on a cellular and on a molecular level in many different organs *in vivo*. However, this method requires suitable deductive approaches to study the mechanisms of neutrophil function in a more simplified environment. *In vitro* and *ex vivo* flow chambers are elegant methods to mimic the inflamed vasculature and allow studying dynamic cell-cell and cell-substrate interactions in high spatiotemporal resolution under controlled conditions. In my presentation, I will introduce the audience into the different *in vivo* and *in vitro* tools to study neutrophil recruitment as mentioned above and discuss the pros and cons of the various approaches.

Keynote 4

Lymph flow maintains the endothelium barrier integrity in lymphatic collecting vessels

Amélie Sabine[1], Cansaran Saygili Demir[1], Esther Bovay[1], Tatiana V. Petrova[1,2]

[1] *Department of Fundamental Oncology, Ludwig Institute for Cancer Research, University of Lausanne, Epalinges, Switzerland*

[2] *Division of Experimental Pathology, Vaud University Hospital Center, University of Lausanne, Lausanne, Switzerland*

The lymphatic vascular network is crucial for body fluid homeostasis, dietary lipid transport and immune surveillance. It consists of two main vessel types: lymphatic capillaries that uptake excess of fluid, solutes and immune cells, and collecting vessels that drain the lymph formed in capillaries to lymph nodes and eventually return it to the blood circulation. Organisation of the cells within the endothelial layer differs drastically between the two vessel types. In capillaries, lymphatic endothelial cells (LECs) are connected with button-like junctions and overlapping flap valves that allow fluid entry into the capillary and prevents its backflow. In collecting vessels, continuous zipper-like junctions between LECs facilitate the transport of lymph. In addition, lymphatic valves regularly positioned along collecting vessels prevent lymph backflow and ensure unidirectional fluid transport. Maintenance of endothelial barrier integrity is crucial for the lymphatic function and impairment of this key feature causes lymphedema, a debilitating disease characterised by chronic swelling, tissue fibrosis and defective immune function. By combining the use of genetic mouse models, high-resolution whole-mount imaging and *in vitro* models of lymphatic endothelial cells subjected to *in vivo*-like flow conditions, our work has established lymph flow as a crucial regulator of lymphatic collecting vessel identity and endothelium barrier integrity, and identified impaired mechanotransduction as an important underlying cause of lymphedema and lymphatic-related pathologies.

Keynote 5

Impact of shear stress on endothelial function

Henning Morawietz

University of Technology Dresden, Germany

tba

Keynote 6

Bioimaging tools in experimental medicine

Steffen Dietzel

Core Facility Bioimaging, BioMedical Center, LMU Munich, Germany

tba

Keynote 7

Flow induced polarization of endothelial cells

Julia J. Mack

Department of Medicine, Division of Cardiology, UCLA, Los Angeles, CA, USA

In the presence of high laminar shear stress, endothelial cells elongate in the direction of blood flow. Are the two ends of the elongated cell, the 'front' and 'rear' as defined by the direction of flow, functionally different? Utilizing the ibidi pump system and high resolution imaging techniques, we visualized plasma membrane-associated proteins and calcium activity in live human aortic endothelial cells under flow. Mapping the distribution of plasma membrane-associated proteins, we observed certain proteins polarize to different ends of the cell. Specifically, we observed localized calcium activity and plasma membrane caveolae asymmetrically distributed. Using atomic force microscopy, we measured the plasma membrane properties to find that they are different at the two ends. Our data suggests that endothelial cell alignment and elongation induces the asymmetric distribution of specific plasma membrane-associated proteins and defines the planar polarization of the cell. We are continuing to explore endothelial cell polarization and plasma membrane properties as a function of flow to understand the compartmentalization of signaling events that support key biological processes in the vascular system.

Keynote 8

Microvesicle-endothelial interactions under shear stress may provide clues for blood vessel pathophysiology

A.P. Kiprianos[1], M. Lang[1], D. Calay[1], E. Tombetti[2,3], G. Wilson[1], D.O. Haskard[1], J.C. Mason[1]

[1] *Vascular Sciences Unit, Imperial Centre for Translational & Experimental Medicine, National Heart and Lung Institute, School of Medicine, Imperial College London, UK*

[2] *Università Vita-Salute San Raffaele, Milan, Italy*

[3] *Department of Biomedical and Clinical Sciences "L. Sacco", Milan University, Milan, Italy*

Cell-derived microvesicles (MV) are heterogeneous sub-cellular bodies released homeostatically and upon activation of parent cells, with which they share lineage similarities. MV are raised in clinical disease, often thought of as markers of cellular and tissue pathophysiology.

MV interactions with the endothelium were modelled to gain clues of their relevance *in vivo*. We characterised MV profiles in patients, and investigated their generation and functional effect on endothelial cells (EC) under flow shear stress using FACS, immunoblotting, confocal microscopy and qRT-PCR.

Endothelial-derived MV (EMV) are differentially regulated by pro-inflammatory mediators offering a distinct phenotype under static, laminar and oscillatory shear stress conditions. Under static conditions, EMV from TNFα-treated cells increase expression of pro-inflammatory genes in naïve EC, while flow substantially challenges and alters both the EMV themselves and the recipient EC response. Overall, shear-stress exerts powerful responses from EC, evolving our understanding of how MV interact with the vasculature.

Keynote 9

How the endothelium drives leukocyte transendothelial migration

Jaap van Buul

University of Amsterdam, The Netherlands

tba

Keynote 10

Leukocyte navigation in 3D microenvironments

Jörg Renkawitz

Ludwig Maximilians University of Munich, Germany

Leukocytes efficiently migrate through diverse microenvironments to execute their effector functions during innate and adaptive immune responses. These microenvironments are crowded by cells and extracellular matrix, generating mazes of differently sized spaces typically smaller than the diameter of the migrating cell. Most mesenchymal and epithelial cells and some but not all cancer cells actively generate their migratory path using pericellular tissue proteolysis. On the contrary, amoeboid cells such as leukocytes typically employ non-destructive strategies of locomotion, raising the question how these extremely fast cells navigate through dense tissues. We recently discovered that leukocytes sample their immediate vicinity for large pore sizes and are thereby able to choose the path of least resistance. This allows them to circumnavigate local obstacles while effectively following global directional cues such as chemotactic gradients. Pore size discrimination is facilitated by frontward positioning of the nucleus, thus enabling the cells to use their bulkiest compartment as a mechanical gauge.

Keynote 11

Cultivation and high-quality imaging of cell aggregates under flow

Miriam Balles, Michael Bergmeier, Roman Zantl, Jan Schwarz

ibidi GmbH, Gräfelfing, Germany

In recent years more and more examples showed higher *in vivo* relevance of 3D cell-based assays compared to 2D assays. In this context, organoid and spheroid-based assays gained vastly in importance. However, spheroid and organoid generation, their nutrient supply in combination with high quality imaging over a long period of time remains challenging, limiting their use in high throughput applications.

We developed a perfusable channel system with numerous homogenously distributed cell aggregate adhesion sites on a highly passivated coverslip. In this system spheroids can either be directly generated by simple injection of cells using a self-sorting process or preformed spheroids or organoids can be homogenously tethered in a large number. The quality of the imaging bottom and the local tethering of numerous cell aggregates enable high resolution fluorescence microscopy with high throughput combined with defined shear stress, metabolite analysis, toxicological screenings or even co-culture of multiple spheroid types.

Talk 1 | Poster 22

A new microfluidic model that allows monitoring of complex vascular structures and cell interaction in a 3D biological matrix

Christian van Dijk, Caroline Cheng

UMC Utrecht, The Netherlands

Here we present a novel microfluidic model that allows endothelial cell (ECs) interaction with supporting pericytes and extracellular matrix (ECM), in a 3D vessel structure subjected to hemodynamic flow, enhancing the complexity to mimic natural conditions. A 3D fibrinogen gel containing supporting pericytes and open channels which enable ECs to form a functional monolayer. Flow was introduced via an ibidi pump system while the dimensions of the design facilitated clear 3D confocal imaging. In our system, ECs interact with pericytes and create a human cell derived blood vessel that maintains an open and perfusable lumen. The artificial blood vessels in this microfluidic system allowed circulation of monocytes, showing natural interaction with immune cells in response to TNFα induced inflammation. The flexibility of this model allows researchers to study specific cell-cell and cell-ECM interactions using different stimuli to mimic (disease) environments while being perfused.

Talk 2 | Poster 23

Maternal platelet interactions with fetal extravillous trophoblast cells

J. Guettler[1], D. Forstner[1], O. Nonn[1], S. Maninger[1], N. Kupper[1], G. Cvirn[2], G. Marsche[3], B. Huppertz[1], M. Gauster[1]

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[2] *Division of Physiological Chemistry, Otto Loewi Research Center, Medical University of Graz, Austria*

[3] *Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Austria*

During early human pregnancy uterine spiral arteries are blocked. Consequently, maternal blood is restrained from entering the intervillous space. However, immunohistochemistry showed platelet staining between extravillous trophoblast and on placental villi during first trimester. Therefore, we investigated the interaction of maternal platelets with extravillous trophoblasts (EVTs) and their marker HLA-G. Immunohistochemical staining with platelet marker CD42b clearly localized platelets between EVTs and on villous trophoblast of first trimester placenta. The platelet-EVT adhesion flow experiment with HLA-G did not show an increased adhesion in comparison to the controls whereas with ADP an increased adhesion was visible. Also, HLA-G did not have an increasing effect on aggregation. This suggests that maternal platelets, in contrast to other circulating maternal blood cells, enter the intervillous space prior to trophoblast plug disintegration and could therefore act as an underappreciated source of inflammation. However, HLA-G does not seem to have an activating effect on human platelets.

Talk 3 | Poster 24

A human blood-brain-barrier *in vitro* model to investigate the role of FOXF2 in cerebral small vessel disease

Judit González-Gallego[1,2], Joseph Kroeger[1,2], Isabel Weisheit[1,2], Julien Klimmt[1,2], Katalin Völgyi[1,3], Martin Dichgans[1,3,4]*, Dominik Paquet[1,4]*

[1] *Institute for Stroke and Dementia Research (ISD), Klinikum der Universität München, Feodor-Lynen-Straße 17, D-81377 Munich*

[2] *Graduate School of Systemic Neurosciences (GSN), Munich, Germany*

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** Senior co-authors*

The human Blood-Brain-Barrier (BBB), composed of brain microvascular endothelial cells, astrocytes and pericytes or smooth muscle cells, is responsible for regulating the transport of substances into the brain. Most of the current BBB in vitro models are based on a two-dimensional monolayer of cells that fail to reproduce a characteristic tubular morphology naturally observed at the human BBB. Here we propose a 3-dimensional BBB model using organ-on-a-chip technology to better recapitulate the anatomical complexity of the human BBB.

Using microfluidics chambers, we generated a fully human iPSC-derived BBB model formed by endothelial cells, mural cells and astrocytes. The endothelial cells formed tight monolayers expressing specific brain vasculature markers such as VE-Cadherin, PECAM-1 and Cadherin-5. Moreover, we show that co-culture of the three cell types induces more complex and interconnected tubular structures.

Talk 4 | Poster 25

Cell culture models to study endothelial activation under septic conditions

T. Eichhorn[1], S. Rauscher[2], C. Hammer[1,2], M. Gröger[2], M.B. Fischer[1,3], V. Weber[1]

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Endothelial activation is a key pathomechanism of sepsis and associated with excessive recruitment and adhesion of leukocytes. We established cell culture models to monitor endothelial activation induced by lipopolysaccharide (LPS)-stimulated blood or septic blood and assessed the effect of cytokine adsorption on endothelial activation. Plasma was diluted with cell culture medium and was used to stimulate endothelial cells under static conditions or under flow. The effect of cytokine adsorption on endothelial activation was studied by pre-treating LPS-stimulated blood or septic plasma with adsorbents. Plasma from LPS-stimulated whole blood and from sepsis patients triggered endothelial activation and induced monocyte adhesion. Pre-treatment with adsorbents efficiently reduced cytokine levels and attenuated endothelial activation. Plasma samples from sepsis patients with comparable IL-6 and TNF-α levels differed regarding their potential to induce endothelial activation. The flow model allows to study the effect of cytokine modulation and to assess the interaction of activated endothelial cells with monocytes.

Talk 5

Shear stress enhances stem cell characteristics, stratification and barrier function in human corneal epithelial cells

Sophia Masterton[1], Mark Ahearne[2]

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[2] *Department of Mechanical and Manufacturing Engineering, Trinity College Dublin, Ireland*

The corneal epithelium is the outermost layer of the cornea containing a limbal stem cell niche. Shear stress is imparted on the corneal epithelium by blinking, the effect of this is poorly understood. This study examines how shear stress affects differentiation and barrier function of limbal stem cells. Cells were seeded at 500,000 cells/cm^2 onto a µ-Slide. Shear stress was applied for 1 and 3 days at 0.122 Pa (low) and 0.243 Pa (high) using a fluidic unit and pump system (ibidi) with a static control also. A significant upregulation of stem cell markers after 1 day low shear suggests that this shear stress rate could be used in the *ex vivo* cell culture of limbal stem cells, enhancing their use in transplantation by upregulating stem cell marker genes. 3 days high shear increased barrier function and stratification, which may be used as an *in vitro* model for the corneal epithelium.

Talk 6

Dual chamber design for accessible, bidirectional perfusion of whole blood vessels

Julia Tarnick[1], Reinhard Tarnick[2], Jamie Davies[1]

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[2] *Independent researcher, Schenkendoebern, Germany*

Lack of blood flow is a major limitation for three-dimensional cell- and organ-culture systems, as the oxygen and nutrient availability can be low in the core of 3D structures. The co-culture of 3D structures with perfused blood vessels bears potential to vascularise 3D cell aggregates *in vitro*. However, traditional blood vessel perfusion systems often allow only unidirectional perfusion or are closed in a way that makes manipulation of blood vessels during the culture period difficult. We have developed a dual chamber perfusion bioreactor which enables the bidirectional perfusion of two blood vessels, similar to the blood flow through arteries and veins *in vivo*. The design further allows easy manipulation such as pro-angiogenic stimulation or co-culture of vessels with different cell types. Currently we are working towards optimizing the system to vascularise kidney organoids *in vitro*.

Talk 7

Increased vascular permeability of human iPSCs-derived endothelial cells with mutation in HNF1A used as a model of maturity-onset diabetes of the young

Neli Kachamakova-Trojanowska[1], Jacek Stepniewski[2], Jozef Dulak[1,2]

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[2] *Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30-387 Krakow, Poland*

Patients with HNF1A-maturity-onset diabetes of the young (MODY) often develop endothelial dysfunction and related microvascular complications, like retinopathy. As the clinical phenotype of HNF1A-MODY diabetes varies considerably, we used human induced pluripotent stem cells (hiPSCs) from two healthy individuals (control) to generate isogenic lines with mutation in HNF1A gene. Subsequently, control hiPSCs and their respective HNF1A clones were differentiated toward endothelial cells (hiPSC-ECs). Human iPSC-ECs from all cell lines had similar expression of CD31 and Tie-2. All tested hiPSC-ECs showed an expected angiogenic response regardless of the mutation introduced. Isogenic hiPSC-ECs responded similarly to stimulation with pro-inflammatory cytokine TNF-α with the increase in ICAM-1 and permeability, however, HNF1A mutated hiPSC-ECs showed higher permeability in comparison to the control cells. Summarizing, both mono- and biallelic mutations of HNF1A in hiPSC-ECs lead to increased permeability in response to TNF-α in normal glycemic conditions, which may have relevance to HNF1A-MODY microvascular complications.

Talk 8

Analyses of autologous endothelial colony forming cells under different shear stress applications

Xenia Kraus[1], Michael Pflaum[2], Stefanie Thoms[1], Rebecca Jonczyk[1], Cornelia Blume[1], Bettina Wiegmann[2], Thomas Scheper[1]

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[2] *Department of Thoracic, Transplant and Cardiovascular Surgery, Hannover Medical School, Hannover, Germany*

A homogenously autologous endothelialized TEVG is crucial to reduce thrombogenicity and inflammatory responses of the patient. It was the aim of the current project to evaluate the role of endothelial progenitor cells (EPC) out of peripheral blood as suitable autologous cell source to cultivate TEVGs in a physiological experimental approach, suitable for clinical applications as advanced therapy mediated product. Mononuclear cells were isolated out of 20 mL peripheral blood of 4 different donors. Endothelial colony forming cells (ECFC) were sub-cultivated out of the mixed EPC-population. ECFCs were cultivated on 0.4 Luer Slide under either laminar or pulsatile flow conditions optimizing cell maturation and monolayer development. Different grades of shear stress were applied (5 dyn versus 20 dyn) to reach connective cell layers. The influence of various mechanical stimuli on the ECFCs was evaluated by measuring gene transcription profiles of shear stress induced marker Thrombomodulin.

Talk 9 | Poster 26

Smooth muscle cell migration and polarization in response to vascular injury

Shiemaa Khogali, Michelle Bendeck

Department of Laboratory Medicine and Pathobiology, University of Toronto
Toronto, ON, Canada

Smooth muscle cells (SMCs) migration and proliferation contribute to the intimal thickening observed in vascular pathologies. In migrating cells *in vitro*, the microtubule-organizing centre (MTOC) is located in front of the nucleus (front-polarized). But in response to injury *in vivo*, neointimal SMCs are rear-polarized. We do not know how rear polarization is induced. Following injury, SMCs migrate to reach the intima where they experience shear stress imposed by blood flow. We hypothesize that shear stress induces MTOC rear polarization in migrating SMCs. Methods/results: Post-confluent layers of medial SMCs were subjected to a wound healing assay and incubated under static or flow conditions (12 dyn/cm^2) in parallel plate flow chambers for 0-24 hours. Cells were fixed and stained for MTOC, microtubules, and nuclei. Confocal microscopy analysis showed a significant increase in rear-polarized cells under flow (56%) compared to static conditions (40%). Conclusion: shear stress induces rear polarization in migrating SMCs.

Talk 10

Treatment of *Staphylococcus aureus* biofilms under flow using ultrasound and vancomycin-targeted microbubbles

Joop J.P. Kouijzer[1], Kirby R. Lattwein[1], Inés Beekers[1], Mariël Leon-Grooters[1], Nico de Jong[1,2], Antonius F.W. van der Steen[1,2], Alexander L. Klibanov[3], Willem J.B. van Wamel[4], Klazina Kooiman[1]

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[3] *Cardiovascular Division, Department of Medicine, University of Virginia, Charlottesville, Virginia, United States of America*

[4] *Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands*

Cardiac device-related biofilms are life-threatening and difficult to treat bacterial infections. Extraction of the infected device is often a major undertaking. Therefore, a specific and less invasive treatment is desperately needed. In this study, we aimed to develop vancomycin-targeted microbubbles and demonstrate their potential for ultrasound-mediated treatment. *Staphylococcus aureus* biofilms were cultivated in plasma-coated IbiTreat µ-slides under flow (5 dyn/cm^2). The adherence of vancomycin-targeted microbubbles to biofilms, under both static and physiological flow conditions (1.5-12.0 dyn/cm^2), was significantly higher in comparison with control microbubbles. Biofilms were treated under flow (5 dyn/cm^2) with vancomycin-targeted microbubbles in combination with ultrasound at 2 MHz, ranging from 250-400 kPa and 5000-10000 cycles. Confocal microscopy recordings revealed up to 27.6% biofilm reduction along the displacement trajectory of the microbubble. These results demonstrate the potential of vancomycin-targeted microbubbles to treat biofilm infections. This study was financially supported by the ERC starting grant 'BUBBLE CURE' (No 805308).

Talk 11 | Poster 27

Engineered skeletal muscle from human induced pluripotent stem cells: potential of flow-based muscle modeling to study neuromuscular diseases

Masatoshi Suzuki

Department of Comparative Biosciences and Stem Cell and Regenerative Medicine Center, University of Wisconsin, Madison, WI, USA

Human induced pluripotent stem cells (iPSCs) present exciting opportunities to study disease processes *in vitro*. Advances in bioengineering allow us to differentiate cells in a system more relevant to their native environment in order to observe naturally occurring phenomena. My laboratory has explored a variety of bioengineered cell culture systems (2-D and 3-D) to promote skeletal muscle differentiation from healthy and patient-derived human iPSCs. Our recent approaches can successfully simulate normal muscle development and specific cellular pathology in the process of neuromuscular disorders such as Pompe disease (glycogen storage disease type II), amyotrophic lateral sclerosis (ALS), and muscular dystrophy (Hosoyama et al., Stem Cells Transl. Med., 2014; Jiwlawat et al., Differentiation, 2017; Jiwlawat et al., Biotechnol. Bioeng., 2019; Lynch et al., Dis. Model Mech., 2019). In this presentation, I will introduce our research progress and discuss the possibility of using flow-based cell culture to improve skeletal muscle modeling.

Talk 12 | Poster 28

Interrogating the biochemical status of cells under flow using Raman-laser-trapping, an innovative analytical tool for cell analysis

H.K. Yosef[1], R. Kronstein-Wiedemann[2], T. Tonn[2], K. Schütze[1]

[1] *CellTool GmbH, Tutzing, Germany*

[2] *Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany*

Raman Trapping Microscopy (RTM) has emerged as a sensitive bioanalytical tool for live cell analysis. We have developed a Raman system with a highly stable laser trapping capability. This setup allows physical trapping of floating cells and particles in solutions and cultures using a highly focused laser beam, facilitating the analysis and sorting of human cells, bacteria, and extracellular vesicles in solutions. Raman results provide a photonic fingerprint and chemical-structural information of the analyzed cell, which can be used to detect induced-subcellular changes in response to stimuli or environmental conditions. To demonstrate this strong potential, RTM was implemented to detect the shelf time for blood bags by monitoring structural changes of hemoglobin in erythrocytes over time, using a small volume of erythrocytes (around 50 µL - ibidi µ-channel slide). Other promising applications of RTM are sepsis, stem cell research, cancer diagnosis, and quality control of cell products.

Poster 1

Brain microvascular heterogeneity: implications for blood brain barrier modeling

Monique F. Stins[1], Andres F. Villabona-Rueda[1,2], Amanda Dziedzic[1], Anne Jedlicka[1], Carlos A. Pardo-Villamizar[3]

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[2] *Johns Hopkins, Department of Internal Medicine, Baltimore, MD, USA*

[3] *Johns Hopkins School of Medicine, Department of Neurology, Baltimore, MD, USA*

Various brain pathologies show striking differences in the white matter (WM) versus grey matter (GM). Possibly, a heterogeneous brain vasculature is responsible for these differential pathologies. Therefore, we isolated vessels from WM and GM brain areas. Microarray analysis revealed enrichment of 813 genes in WM versus GM ($p<0.05$), including transporter proteins, junctional molecules, cell adhesion molecules, immune response proteins and hemostasis/ coagulation molecules. These differences have implications for signaling responses of the WM versus GM BBB endothelium, including differential responses to flow. Currently, *in vitro* models of the blood brain barrier (BBB) are based on GM endothelium or inducible progenitor cells (iPC). However, it is unclear whether iPC models represent true GM or WM BBB-endothelium or epithelial cells. To understand underlying mechanisms and specific patterns of neurovascular diseases differentially affecting WM versus GM, improved BBB-models that better represent the endothelium of interest, are needed, including the application of flow.

Poster 2

Cell cultivation under flow: the food toxicology perspective

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From the toxicological perspective, cell cultivation under flow is very relevant for compounds reaching the endothelium via the bloodstream, but it can be crucial also in other cases. Particularly, intestinal and bladder cells can experience shear stress stimulation due to the movement of the luminal fluids, albeit being exposed to xenobiotics and their metabolites. This scenario is plausible in the study of food contaminants, and we investigated the cross-talk between shear stress and food-borne toxicants at intestinal (HT-29 and HCEC cells1) and bladder level (T24 cells). This approach allowed us to study the compounds of interest in more physiological environment and enabled the description of novel mechanisms that otherwise would have been difficult to grasp by classical *in vitro* approach.

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Poster 3

High throughput, label-free, optical characterization and sorting of particles and cells

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Despite the progress made in recent years in droplet-based microfluidics, it is still in a developmental stage. It must permit the integration of multiple laboratory functions into one single chip. Therefore, we developed ODIN, a high performance label-free optical sensing system for real-time detection, analysis and sorting of biological and synthetic particles and complex structures in a continuous flow. ODIN automatically characterizes particles by size, shape, morphology, brightness, speed and many other parameters. The result is available immediately and a trigger signal is set if predefined conditions are met which enables sorting of defined populations, tailor-made for every experiment. We show measurements where empty droplets and droplets containing a cell are separated by a microfluidic switch triggered by the ODIN device. Additionally, a mixture of droplets with different sizes is sorted with unrivaled specificity, selectivity and throughput.

Poster 4

Drug interactivity studies to define synergistic anti-malarial combinatorial regimes for emetine dihydrochloride

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The emergence and spread of artemisinin resistance to *Plasmodium falciparum* poses serious threat to ongoing malaria control efforts. The development of treatments through drug repositioning may offer novel candidates permitting new combinatorial regimes with existing anti-malarials. Drug repositioning screens previously carried out in our laboratory reported the emetine has a potent antimalarial efficacy (IC50 47nM for *P. falciparum* K1 strain). We present here the preliminary data from a study designed to define the combinatorial therapeutic potential of emetine with a panel of antimalarial drugs, in a bid to minimise non-target effects previously experienced with the use of the drug in amoebiasis. All combinations were analysed using the optimised CalcuSyn fixed-ratio method. Our preliminary data identified AN16 as the combinatorial partner drug displaying maximum synergistic interactivity with emetine. The MTT cytotoxicity results indicated that the emetine-AN16 combination has a better selectivity index comparing to emetine. Further investigation is recommended.

Poster 5

Establishment of a novel flow culture system to examine the interplay between mother and placenta

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During pregnancy, the placenta releases extracellular vesicles (EVs) into the maternal circulation. In pregnancy pathologies such as preeclampsia the release of placental EVs is altered leading to damage and activation of the maternal vascular system. We developed a novel flow-system simulating diverse health conditions of pregnancy to analyze the effect of EVs released from the placenta on maternal endothelial cells. Placental explants from third trimester placentas were cultured in a flow-system (QV500 Kirkstall + TEB500, Ebers) and compared to explants cultured under commonly used static conditions (6-well plates). Scanning electron microscopical analysis revealed different appearances of tissues cultured under flow or static conditions. EVs were isolated and analyzed using nanoparticle tracking analysis, structured illumination microscopy and transmission electron microscopy. Next, adult primary endothelial cells will be stimulated with isolated placental EVs to simulate the *in vivo* situation of blood flow from the placenta to the mother and back.

Poster 6

Barrier integrity of HUVEC cultured in 3-lane Organoplate® with low level of oxygen

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The placenta is a temporary organ, composed of endothelial and epithelial cells. It forms a barrier between mother and fetus and regulates fetal development by controlling gas exchange and transfer of nutrients and waste. This development is guided by hypoxic environment. In this study we simultaneously cultured perfused tubules of Human Umbilical Vein Endothelial Cells (HUVEC) in a 3-lane microfluidic chip. We investigated the effect of hypoxic condition on the barrier function by using two different approaches. The apparent permeability (Papp) of the tubule for a fluorescent compound was determined using an imaged based approach. OrganoTEERTM was used to determine the Transendothelial electrical resistance (TEER) of the tubules. HUVECs were cultured for 3 days in Organoplate® against extracellular matrix under normal oxygen concentration. To simulate hypoxic conditions, the culture was maintained at 5% oxygen for 24 h. We observed a significant effect of hypoxia on the integrity of the barrier.

Poster 7

LifeGel, a general purpose hydrogels for 3D cell culture

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3D cell cultures became already a valuable research model but still the majority of the research both in industry and academia is being done on standard 2D cell cultures. LifeGel is a technology that enables the researcher to customize the hydrogel for specific application. LifeGel is a protein based hydrogel, growth factors-free, repeatable and scalable. LifeGel has been adapted to different research models like: pancreatic cancer tumor formation model on murine and human cell lines (respectively Pan_02 and PANC-1), breast cancer tumor formation model (MDA-MB-231), melanoma (B16) and other cancer cell lines. LifeGel is also suitable for angiogenesis test (HUVEC) and toxicology research. We have customized the LifeGel for the spheroid formation of L929 cells and spheroid growth of CHO cells. We are testing LifeGel for new applications like iPS growth, viral lung infection models, animal embryos development, murine primary hepatocytes and pig MSC.

Poster 8

The role of mechanosensitive protein Piezo2 in pulmonary hypertension

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Piezo proteins are machanosensitive ion channels that transfer mechanical stimuli to cellular responses. High shear stress (HSS) is a risk factor in the pathogenesis of pulmonary hypertension (PH). However, whether the Piezo2 can play a role in PH is currently unknown. Human lung microvascular endothelial cells (MVECs) were cultured in the ibidi system. The shear stress was increased every 24 hours following either a normal shear stress (NSS: 8,15, 21 dyn/cm^2) or HSS (8,21,67 dyn/cm^2) profile. After 72 hours the gene expression of MVECs was compared with that of MVECs in static culture conditions. The expression of Piezo2 was significantly lower in the static MVECs as compared to the MVECs exposed to NSS. Furthermore, in MVECs exposed to HSS, piezo2 expression was lower as compared to both NSS and static conditions ($P<0.01$). Our data suggests that the gene expression of Piezo2 is regulated by the shear stress under normal physiological conditions.

Poster 9

Influence of aging of the quality and dynamics of mouse cardiac fibroblast in culture

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Cardiac aging is a multifactorial process characterized by a gradual decrease in physiological functions at the molecular and cellular level. Cardiac fibroblast plays an important role in maintaing the structural integrity of the heart trough regulation and turnover of the extracellular matrix Ageing affects cardiac function in multiple manners. Here we analyzed several phenotypic and molecular features of heart-derived cFb in culture. Comparative analysis of cells isolated from young (2 mo-old) and old (16-mo old) mice revealed different kinetics of cells with passaging, as well as different metabolic potential. Furthermore, changes in the expression of extracellular matrix components (including collagens and fibronectin), α-SMA and TGFβ have been noticed in cFb derived from aged hearts, as compared to young hearts. Taken together, our data showed that cFb culture is affected by natural ageing of the organism they are derived from, through important changes both in the quality and dynamics of cells.

Poster 10

Designing a miniaturized *ex-vivo* ECMO-circuit with the ibidi Pump System

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How blood components interact with polymethylpentene (PMP) gas exchange fibers (GEF) of an extracorporeal membrane oxygenation system (ECMO) is not completely understood. The aim was to enable live monitoring of protein adhesion to PMP in a microfluidic chamber under flow. A single GEF was inserted vertically into a 0.4µ-Luer-ibidi Slide. Human plasma/ serum samples were prepared from anticoagulated whole blood by centrifugation. An ibidi perfusion set (yellow-and-green) was primed with saline, then filled with plasma/ serum. Protein adhesion to the GEF was then monitored under flow. Plasma, but not serum, grew thrombotic deposits of various extent around the GEF. Unsteady flow conditions during valve-switching partially ripped off thrombotic material. Furthermore, in elbow luer connectors and at the GEF, shear stress reached 50-100 dyn/cm^2, exceeding expected shear stress ranges (5-35 dyn/cm^2). Shearing-effects and flow irregularities need to be considered, when microfluidic chamber experiments are performed with ibidi Pump Systems.

Poster 11

Breast tumor on chip

Subia Bano

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Breast cancer is the most common invasive cancer among women. There are several chemotherapeutic and radiotherapeutics approaches are available but they have certain limitations. Over the past few years, improved understanding of the microenvironment heterogeneity of breast cancer has allowed the development of more effective treatment strategy. However, still researchers are unable to recapitulate the entire tumor microenvironment to study the tumor progression and invasion. In this direction, breast tumor-on-chip has emerged as an alternative model to study the tumor microenvironment and deciphering its role in metastasis. In this work the multi-compartment microfluidics platform is generated by co-culturing of cancer cells, fibroblast and endothelial cells into biocompatible hydrogel to produce a multi-organ-on-a-chip device. It recapitulates tumor microenvironment and vascularized channel between the compartment mimic organ-like function. The implementation of microfluidics system into breast tumor will assist more efficient testing platform in the current therapeutic approach.

Poster 12

Microchip to host a mini CNS with a muscle for pharmacology

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Neurochips are practical tools for understanding neuronal communication, plasticity together with drug testing and discovery. Implementation of microfluidics into blood brain barrier (BBB) provides more precise fluidic flow control and assessment. Current brains on a chip are only restricted to growing different neurons types as astrocytes to mimic the BBB and do not connect to the periphery. Here we incorporate a muscle to the 3D chip in order to investigate the motor pathway under perturbation. Axon cue guidance allows neurons to functionally bind to muscles. Further plans consist in multi electrode array integration and flow control. This technology is highly favorable as it permits to cure diseases and *in situ* implants. The focus is on solving Parkinson. The advantage of this system reduces cost of animal testing by providing new tools in current treatment strategies.

Poster 13

Design of protein membranes for suspended cell culture in lung-on-chip devices

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The lung is the main organ of respiration and facilitates gas exchange across the alveolar-capillary (air-liquid) interface. Essential contributions from tensile and shear stress to cell fate and function are largely overlooked in lung models. Significantly, altered mechanical stiffness is a hallmark of many lung conditions, and is directly related to concomitant changes in the composition and/or integrity of fibrous matrix protein networks. In this study, membranes were designed from protein blends to create substrates with more physiologically relevant biological and mechanical properties for cell culture. Membranes were suspended in a variety of microfluidic platforms, enabling the application of external pressure during culture, towards investigating the effect of soft substrates, flow and tensile stress on cell proliferation and barrier function. These studies have the potential to define lung-on-chip devices with both physiological and disease-matched mechanical profiles for drug testing.

Poster 14

Mesangial specific CD248 expression controls a novel intercellular cross-talk within glomeruli

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Aims: CD248 is a pericyte-specific type I transmembrane glycoprotein, suggesting a potential role in cell-cell interaction that is related to tissue remodeling and repair. Aim of our study is to investigate the role of mesangial CD248 in regulating proliferation and apoptosis on glomerular endothelial cells and podocytes. Results: We detected a marked increase in renal expression of CD248 after 26 weeks in streptozotocin-induced diabetic mice. Knockout of CD248 protected mice from diabetes induced albuminuria, podocyte loss, and glomerular fibrosis. Investigating potential mechanisms *in vitro*, we revealed that shRNA mediated knockdown of CD248 prevents glucose induced UPR and inflammasome activation. We now investigate the effect of pericyte-expressed CD248 on function of barrier-defining cells (podocytes, tubular cells, glomerular endothelia cells). Conclusion: Our findings indicate that mesangial specific CD248 expression controls a novel intercellular cross-talk within glomeruli which governs apoptosis and proliferation of podocytes and glomerular endothelial cells.

Poster 15

The Role of MRP8/14 in leukocyte recruitment *in vivo*

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Myeloid related protein 8/14 (MRP8/14) is a Ca^{2+} binding protein that is actively secreted during leukocyte recruitment, mediating β2 integrin activation and neutrophil adhesion *in vivo*. Despite representing 40% of cytosolic protein content in neutrophils, a putative intracellular role of MRP8/14 remains elusive. Within our study, we investigated leukocyte adhesion and extravasation *in vivo* in WT and $Mrp14^{-/-}$ mice, which are functional MRP8/14 deficient mice and found that the lack of MRP8/14 causes neutrophil adhesion deficiency and impaired extravasation *in vivo*. Next, we analysed WT and $Mrp14^{-/-}$ neutrophil postarrest modification steps in microflow chambers. $Mrp14^{-/-}$ neutrophils showed defective spreading behaviour, were unable to polarize properly, crawled for longer distances and were more prone to detach at increasing shear stress levels. Moreover, phagocytosis was reduced in the absence of MRP8/14. We postulate an intracellular function of MRP8/14 in mediating neutrophil post arrest modifications and effector functions, presumably by coordinating Ca^{2+} signalling.

Poster 16

The voltage-gated potassium channel KV1.3 regulates neutrophil adhesion and crawling *in vitro*

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During pregnancy, the maternal immune system prevents infections and, at the same time, protects the semi-allograft embryo. This fine balance of immune regulation is established by numerous factors and receptors, one factor is the small peptide PreImplantation Factor (PIF). Recently, our group was able to show that PIF binds to the voltage-gated potassium channel KV1.3 on neutrophils, thereby modulating intracellular calcium concentrations. However, the role of this channel during recruitment of neutrophils to sites of inflammation is yet unknown. We investigated the role of KV1.3 using *in vitro* flow chambers. Both, pharmacological inhibition and genetic deletion of KV1.3 increased leukocyte rolling velocities and reduced numbers of adherent leukocytes, a phenotype also observed in leukocytes from pregnant C57BL/6 mice. Furthermore, neutrophils from KV1.3$^{-/-}$ mice exhibited increased crawling distance and velocities under flow, suggesting impaired post-arrest modifications. With this study, we shed new light on how immune suppression during pregnancy might occur.

Poster 17

miR-142 controls the migration of young and old follicular B cells

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miR-142 is highly expressed in hematopoietic cells and has been reported to play diverse roles in these cells, including B cells. Mature follicular B cells patrol the body and are strategically positioned within lymphoid organs to detect foreign antigens. B cell distribution and migration depend on their susceptibility to chemoattractants. Remarkably, aging leads to changes in B cell migratory properties. In agreement, the expression of the chemokine receptors CXCR4 and CCR7 is altered. We are investigating the role of miR-142 in follicular B cell migration in young and aged mice. Absence of miR-142 affected the distribution, surface expression of chemotactic receptors and motility of follicular B cells. Thus, our data suggest that miR-142 is an important regulator of follicular B cell migratory properties and the activity of this miRNA appears to attenuate the effect of aging on follicular B cell migration, in mice.

Poster 18

Genomic and proteomic characterization of a dynamic *in vitro* model of the human vasculature

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Background: The vasculature is a highly specialized organ, lined by organ-specific endothelial cells mediating critical pharmacological aspects or adverse events. *In vitro* models of the vasculature lack essential physiological parameters such as flow-induced shear stress.

Strategy: We present the characterization of a microfluidic *in vitro* platform containing human primary endothelial cells cultured under flow and compare morphological, phenotypical and functional parameters with static cultures.

Results: Human endothelial cells cultured under flow at a shear stress of 10 dyn/cm^2 for 48 h, elongate and align with the direction of the applied flow. Proteomics, secretomics and genomics characterization of cells cultured under flow have highlighted modifications of key markers and regulation of pathways like e.g. cell adhesion, morphology and angiogenesis.

Conclusion: Blood flow-induced shear stress is a major stimulus of endothelium microenvironment, which drives endothelial cells phenotype. We demonstrate that human primary endothelial cells cultured under physiological flow recapitulate key morphological and functional features observed *in vivo.*

Poster 19

In situ protein patterning and hydrogel polymerization to control the micro-environment of cells under flow

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In vivo, the cellular microenvironment has a crucial impact on the regulation of cell behavior and functions such as cellular differentiation, proliferation and migration. One of the challenges confronting cell biologists is to mimic this microenvironment *in vitro* in order to more efficiently study living cells and model diseases. Here, we present PRIMO: a contactless and maskless UV projection system, which allows to control both the mechanical and biochemical properties of *in vitro* microenvironments. We will focus on *in situ* polymerization and patterning capabilities of PRIMO. In fact, PRIMO allows to control cell microenvironment inside commercial or in house microfluidic channels, to allow a better control of cell under flow.

Poster 20

Targets of the microRNA let-7b in mouse and human endothelial cells

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MicroRNAs contribute to endothelial cell (EC) maladaptation in response to disturbed flow and hyperlipidemia. Our previous results suggest that the highly conserved let-7b plays an important role in this regard. To identify the targeting network of let-7b, we captured and identified its targets using RISC-trap approach in mouse and human aortic ECs and sequencing using the Illumina HiSeq2000, respectively. Here we show that although let-7b is highly conserved, most of the RNA targets in mouse and human differ. UHRF2, MLLT10 and the Lincpint lncRNA were among the conserved targets with highly conserved canonical binding sites. Inhibiting let-7b in mouse aortic ECs upregulated Uhrf2, Mllt10 and Lincpint expression. let-7b binding site on human UHRF2 and Mllt10 3'UTR was confirmed by luciferase binding assay ($p<0.05$, Student t test, n=3-5). Our results show that UHRF2 and Mllt10 are targets of let-7b in aortic ECs and are valuable for future studies.

Poster 21

Towards a 3D vascularised microfluidic cell-culture system

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Cardiac remodelling is an adaptive response to functional or structural changes of the myocardium and play an important role in health and disease, including Cardiac sudden Death (CSD) and arrhythmia. Primary electrical remodelling takes place in response to alterations in electrical activation, secondary electrical remodelling follows structural insults such as heart disease such as myocardial infarctum or hypertrophy. One major factor for arrhythmia are the changes in distribution of cardiac gap junctions that allow propagation of the action potential throughput the tissue and synchronise beating. Fibroblasts-cardiomyocites junction and the role of cell heterogeneity in the population are key in this process. We are building a cardiac fibrosis model on-a-chip.

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